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Formulation and Characterization of Inhalable Magnetic Nanocomposite Microparticles (MnMs) for Targeted Pulmonary Delivery via Spray Drying

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Abstract

Targeted pulmonary delivery facilitates the direct application of bioactive materials to the lungs in a controlled manner and provides an exciting platform for targeting magnetic nanoparticles (MNPs) to the lungs. Iron oxide MNPs remotely heat in the presence of an alternating magnetic field (AMF) providing unique opportunities for therapeutic applications such as hyperthermia. In this study, spray drying was used to formulate magnetic nanocomposite microparticles (“MnMs”) consisting of iron oxide MNPs and D-mannitol. The physicochemical properties of these MnMs were evaluated and the *in vitro* aerosol dispersion performance of the dry powders was measured by the Next Generation Impactor®. For all powders the mass median aerosol diameter (MMAD) was < 5 µm and deposition patterns revealed that MnMs could deposit throughout the lungs. Heating studies with a custom AMF showed that MNPs retain excellent thermal properties after spray drying into composite dry powders, with specific absorption ratios (SAR) >200 W/g, and *in vitro* studies on a human lung cell line indicated moderate cytotoxicity of these materials. These inhalable composites present a class of materials with many potential applications and pose a promising approach for thermal treatment of the lungs through targeted pulmonary administration of MNPs.

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Keywords

Magnetic nanoparticles; iron oxide; remote heating; dry powder inhalation aerosols; nanocomposites; spray drying

1.0 Introduction

Targeted pulmonary inhalation aerosol delivery facilitates the direct application of bioactive materials to the lungs in a controlled manner. Medical conditions such as asthma, chronic obstructive pulmonary disease (COPD), and pulmonary infections secondary to cystic fibrosis employ targeted pulmonary inhalation aerosol delivery as a first-line treatment because of inherent advantages [1] such as a fast onset of pharmaceutical action, higher local concentrations of drugs, and reduced systemic side effects [2]. Further, the treatments of pulmonary diseases with inhalation methods avoid first-pass metabolism of pharmaceutical agents [3] resulting in significantly less drug required for aerosol dosage forms to exert a therapeutic effect in the lungs.

Dry powder inhalation aerosol offers several advantages over liquid aerosol formulations such as increased stability of formulations and controllable particle size for targeting regions of the lung [1, 4]. Additionally, dry powder formulations can be used to administer higher amounts of water insoluble pharmaceutical actives as in contrast to liquid-based inhalation aerosol systems such as nebulizers and pressurized meter dose inhalers (pMDIs).

Recently, there has been a growing interest in utilizing the advantages of pulmonary delivery for treating lung diseases such as cystic fibrosis (CF) and lung cancer thereby expanding the potential applications of successful aerosol formulations. Phase I trials of inhaled cisplatin showed no dose-limiting toxicity or maximum tolerated dose[5] and a phase II study of inhaled doxorubicin showed a favorable median survival compared to intravenous administration [6]. Traditionally, inhaled therapies such as these utilize aerosols consisting of small molecule drugs and excipients; however, targeted pulmonary delivery provides a platform for localizing novel multifunctional nanoparticles to the lungs through direct, topical application [3, 7].

The concept of delivering iron oxide MNPs¹ to the lung for drug delivery purposes has been explored recently due to their unique properties that provide novel functionality to drug delivery systems as well as their FDA approval as marketed pharmaceutical products for medical applications such as a contrast agent in magnetic resonance imaging (MRI) [8–18]. One such functionality is the magnetic guidance of aerosols containing MNPs for enhanced targeting to specific regions within the lungs. The Rudolph lab has successfully directed magnetic aerosols, as nebulized MNP suspensions, to regions of the lungs using static magnetic fields [10, 19]. Similar in vitro studies have recently shown promise for magnetic guidance of dry powder composites containing MNPs [11, 16, 17]. These studies show the ability to deliver a higher dose of model drugs to specific regions of the lungs and

¹AMF: Alternating magnetic field; MMAD: Mass median aerosol diameter; MnM: Magnetic nanocomposite microparticle; MNP: Magnetic nanoparticle; NGI: Next generation impactor

demonstrate one of the many potential advantages to incorporating multifunctional MNPs to inhalation therapies.

Additional advantages of using MNP-containing aerosols include their ability to remotely heat in the presence of an alternating magnetic field (AMF) providing enhanced control over actuating the onset of therapy [20–22]. In suspension, MNPs generate heat through frictional (Brownian) and magnetic (Néel) relaxation processes, and the heat generated from these particles can be used to trigger other therapies, increase transport of particles, and induce hyperthermia as a thermal treatment [21, 23–26].

Hyperthermia is the heating of tissue to 41–45°C and can be applied locally, regionally, or throughout the body. Additionally, it has shown promise in treating cancer by enhancing chemotherapy and radiotherapy [27–29]. Locally targeted hyperthermia in the treatment of cancer has the advantage of reducing undesired cellular death to healthy tissue and can be accomplished with MNPs under AMF exposure [8, 30–32]. Previous clinical trials have utilized magnetic nanoparticles under AMF exposure to demonstrate the feasibility of remotely administering interstitial heating to cancer patients [33, 34]. Hence, delivering MNPs directly to the lungs through inhalable carriers could allow for targeted administration of hyperthermia in the treatment of lung cancer provided these carriers have appropriate aerodynamic properties. However, the introduction of foreign matter such as MNPs to the lungs poses safety risks to the patient, thus necessitating toxicological evaluation.

Previous *in vitro* toxicity studies with fluorescent iron oxide MNPs showed no pulmonary toxicity in murine alveolar macrophages for concentrations as high as 1000 µg/ml. However, *in vivo* studies on adult male Wistar rats showed general and organ toxicity after intratracheal administration of iron oxide nanoparticle suspensions at a low and high dose of 1 and 5 mg/ kg, respectively [35, 36]. Additionally, the effect on body weight, as well as liver and kidney weight, indicated systemic spreading of these particles through the systemic circulation.

The objectives of this work are to formulate composite dry powders for inhalation aerosol delivery containing multifunctional nanoparticles that retain their ability to heat and have potential for the future development of novel pulmonary treatments. In this study, spray drying in closed-mode was used to formulate magnetic nanocomposite microparticles (“MnMs”) consisting of iron oxide MNPs and D-mannitol, which is an FDA-approved pharmaceutical excipient shown to be well tolerated for dry powder inhalation [37]. Additionally, a custom Taylor Winfield AMF source was used to evaluate the ability of these composites to remotely heat under AMF exposure. Figure 1 provides a schematic representation of the formulation and applicability of these materials. The physicochemical properties of these inhalable dry powders were thoroughly characterized with a variety of techniques and their *in vitro* aerosol performance was studied with the Next Generation Impactor®. The capability of these materials to remotely heat in the presence of an AMF was examined and the *in vitro* cytotoxicity of these materials was examined in a human lung cell line (A549).

2.0 Material and methods

2.1 Materials

Iron(II) chloride tetrahydrate ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$), iron(III) chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), and D-mannitol were obtained from Sigma Aldrich (St. Louis, MO). Chloroform, anhydrous methanol, and high performance liquid chromatography-grade methanol were purchased from Fisher Scientific (Pittsburgh, PA). Ultrahigh-purity nitrogen gas was from Scott-Gross (Lexington, KY), and ammonium hydroxide (NH_4OH) was obtained from EMD chemicals (Gibbstown, NJ). Honeywell Riedel-de Haen® Hydranal®-Coulomat AD, reagent for Karl Fischer (KF) coulometric titration, was purchased from Sigma-Aldrich (St Louis, MO). Special anhydrous AQUA STAR methanol for moisture determinations was purchased from EMD Chemicals, Inc. (Darmstadt, Germany)

2.2 Synthesis of iron oxide magnetic nanoparticles (MNPs)

Aqueous co-precipitation was used to synthesize iron oxide nanoparticles. Briefly, $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ (Fe^{2+}) and $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (Fe^{3+}) salts were dissolved in 40 mL of deionized (DI) water at a 2:1 $\text{Fe}^{3+}:\text{Fe}^{2+}$ molar ratio. The solution was sonicated in a water bath for approximately 20 min and transferred to a 3-neck 500 mL round bottom flask. The round bottom flask was heated to 85°C under nitrogen flow with a mechanical stirrer at 300 rpm. Once the vessel reached 85°C, 5 mL of NH_4OH (28 % w/w) was added with a syringe and the reaction was carried out for 1 hour. Recovered particles were washed with ethanol via magnetic decantation and dried in a vacuum oven prior to spray drying.

2.3 Formulation of magnetic nanocomposite microparticles (MnMs) with spray drying

Spray drying was accomplished with a Büchi Mini Spray Dryer B290 coupled with a B-295 Inert Loop and high-performance cyclone (all from Büchi Labortechnik, Flawil, Switzerland) using parameters previously optimized by our lab [38]. Spray drying experiments were carried out in closed mode using UHP dry nitrogen gas at a flow rate of 600 L/h, an inlet temperature of 150°C, and an aspirator rate of 35 m³/h. Once the inlet temperature of 150°C was reached, pure methanol was fed to the spray dryer until the outlet temperature was stable and this temperature was recorded, as tabulated in Table 1. For feed mixtures, D-mannitol was dissolved in methanol and iron oxide MNPs were weighed out and subsequently dispersed such that the combined mass of these two components resulted in a dilute concentration of 0.1% wt/vol. The MF20 and MF5 powders resulted in a MNP concentration of 0.20 mg/ml and 0.05 mg/ml, respectively. Two liters of the feed mixture were fed to the spray dryer at a pump rate of 50% (15 mL/min) through a stainless steel nozzle with a diameter of 0.7 mm. For the MF20 and MF5 experiments, which contained MNPs, the feed mixture was sonicated throughout the experiment to insure adequate dispersion of MNPs. Powders were collected and transferred to a glass desiccator over Drierite™ and stored in the freezer at -23°C. Two different iron oxide loadings were incorporated into the powders and Table 1 summarizes these systems and their spray drying parameters.

2.4 Electron microscopy

Scanning transmission electron microscopy (STEM) was carried out in order to confirm the presence of iron oxide in the MnMs and to observe the spatial distribution of the nanoparticles within the composites. Powders were dispersed in chloroform at 100 µg/ml, added dropwise onto a support film grid (Electron Microscopy Sciences, Hatfield, PA), and allowed to dry overnight in a desiccator over Drierite™. Chloroform was used as a dispersant because D-Mannitol is practically insoluble in this organic solvent [39]. Images were captured with a JEOL 2100F Field Emission Electron Microscope (JOEL, Tokyo, Japan) equipped with an Oxford EDS detector (Oxford Instruments, Oxford, United Kingdom).

Scanning electron microscopy (SEM) was performed in order to observe the particle size, surface roughness, shape and morphology. A Hitachi S4300 microscope (Tokyo, Japan) was used to capture images. Double-sided adhesive carbon tabs were adhered to aluminum studs (Ted Pella, Redding, CA) and blotted on weigh paper with our dry powder. These samples were coated with a thin film of a gold/palladium alloy using an Emscope SC400 sputter coating system at 20 mA for 2 minutes under argon gas. For all electron microscopy experiments at least 3 independent samples were examined and multiple images were examined for each sample.

2.5 Thermal gravimetric analysis (TGA)

Thermal gravimetric analysis was performed using a TA TGA Q50 (TA Instruments New Castle, DE) in order to quantify the actual MNP loading in the powders. Samples were placed in a Tzero™ alodined aluminum pan that was hermetically sealed and then placed into the furnace along with an empty alodined aluminum hermetically sealed reference pan. A hole was punched in the lid of the pan in order to allow for evaporation. The temperature of the furnace was raised to 120°C at a rate of 5°C /min and then held isothermally for 20 minutes to remove any trace solvent. The temperature was then increased to 500°C and held isothermally for 30 minutes in order to completely decompose any D-mannitol present and allow for calculating the actual mass of iron oxide nanoparticles in the dry powders. Experiments were carried out in triplicate

2.6 Karl Fischer titration

Karl Fischer coulometric titration was carried out in order to determine the residual water content in the powders. A 737 KF Coulometer coupled with 703 Ti Stand (Metrohm Ltd., Antwerp, Belgium) was used. Approximately 5 mg of these powders were dissolved into anhydrous methanol for moisture determinations and allowed to sonicate for approximately 10 minutes to ensure complete dissolution of composites thereby releasing the MNPs into suspension. The resulting dispersions were injected into reaction cell filled with Hydranal® KF reagent with a 2 mL syringe and the amount of water injected was measured. Pure solvent was also injected to determine the amount of residual water in the solvent. The mass of water in the injected samples was used to calculate the water content in the dry powders by subtracting out the water content of the solvent.

2.7 Differential scanning calorimetry (DSC)

DSC was carried out in order to determine the thermal phase transitions of the dry powders. An Auto Q20 Differential Scanning Calorimeter equipped with an RSC-90 refrigerated cooling system (TA Instruments-Waters LLC, New Castle, DE) and Tzero™ technology was used for measurements. Approximately 2 mg of sample was weighed into a Tzero™ alodined aluminum pan that was hermetically sealed and then placed into the furnace along with an empty alodined aluminum hermetically sealed reference pan. The temperature was initialized at 0°C and subsequently raised to 250°C at a rate of 5.00°C /min.

2.8 X-ray powder diffraction (XRPD)

XRPD was used to examine the long range order of the MnMs. Powders were analyzed by an X-ray diffractometer (Multiplex, Rigaku) with Cu K α radiation (40 kV, 44 mA) in order to examine their crystallinity. Scans were obtained from 5 to 60° 2 θ with step size of 0.2 and scan rate of 2 °/min.

2.9 Particle size analysis

Dynamic Light Scattering (DLS) was performed to determine the hydrodynamic diameter of the MNPs before spray drying and after being released by dissolution of the MnMs. Aqueous dispersions were made with a MNP concentration of 200 μ g/mL and were read in a disposable cuvette using a Beckman Coulter® Delsa Nano Submicron Particle Size and Zeta Potential Particle Analyzer (Beckman Coulter, Brea, California). For these experiments, MNPs from the same synthesis batch were compared in order to examine the effects of spray drying on particle diameter and avoid observing batch-to-batch variability as seen in precipitation reactions.

SigmaScan® 5.0 software (Systat, SanJose, CA) was used to analyze SEM images and digitally determine the mean size and standard deviation of MnMs. Representative SEM images at a magnification of 5000 $\times$ were analyzed by measuring the diameter of at least 100 particles per image with the SigmaScan software.

2.10 In vitro aerosol dispersion performance with the Next Generation Impactor™ (NGI™)

Impactor studies were carried out with a M170 Next Generation Impactor™ (MSP Corporation Shoreview, MN) in accordance with United States Pharmacopeia (USP) Chapter <601> specifications on aerosols [40]. The conditions for these experiments were previously reported [41, 42]. The NGI was equipped with a stainless steel induction port (USP throat adaptor) attachment and specialized stainless steel NGI™ gravimetric insert cups (NGI Model 170, MSP Corporation, Shoreview, Minnesota). Type A/E glass fiber filters with diameter 55mm (Pall Corporation, NY) were placed on each stage of the impactor and weighed before and after the experiment to determine the particle mass deposited. Approximately 10 mg of powder was loaded into each clear hydroxypropyl methylcellulose, size-3 capsule (Quali-V®) (QUALICAPS, Spain) and these capsules were placed into a HandiHaler® DPI™ device (Boehringer Ingelheim Pharmaceuticals, Inc. Ridgefield, CT) attached to a customized rubber mouth piece connected to the NGI™. Three capsules were loaded and released for each sample and experiments were performed in triplicate. The total dose for each sample was approximately 30 mg. A Copley HCP5 vacuum pump (Copley

Scientific, United Kingdom) and a Copley TPK 2000 critical flow controller (Copley Scientific, United Kingdom) were used to produce a flow rate (Q) of 60 L/min in order to model the flow rate in a healthy adult lung. This flow rate was adjusted before each experiment using a Copley DFM 2000 flow meter (Copley Scientific, United Kingdom).

For Q=60 L/min, the aerodynamic cutoff diameters (D_{a50}) for each stage of the impactor were given from the manufacturer as: stage 1 (8.06 μm); stage 2 (4.46 μm); stage 3 (2.82 μm); stage 4 (1.66 μm); stage 5 (0.94 μm); stage 6 (0.55 μm); and stage 7 (0.34 μm). The emitted dose (ED), respirable fraction (RF), and fine particle fraction (FPF) were calculated as follows:

$$\text{ED \%} = \frac{\text{Initial mass in capsules} - \text{Final mass in capsules}}{\text{Initial mass in capsules}} \times 100\% \quad (1)$$

$$\text{RF \%} = \frac{\text{Mass of particles on stages } 2 - 7}{\text{Total particle mass on all stages}} \times 100\% \quad (2)$$

$$\text{FPF \%} = \frac{\text{Mass of particles on stages } 2 - 7}{\text{Initial mass in capsules}} \times 100\% \quad (3)$$

The mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) for the particles were determined using a Mathematica® program written by Dr. Warren Finlay [43].

2.11 Alternating magnetic field (AMF) Heating studies

Heating studies were performed on synthesized MNPs as well as MNPs released from MnMs upon aqueous dissolution using a custom Taylor Winfield AMF source (Taylor-Winfield Technologies, Youngstown, Ohio). Pure MNPs (as synthesized) and MnMs were dissolved at a concentration of 10 mg/ml. Particles were dispersed in DI water, sonicated for approximately 20 min and placed in an oven at 25°C. 1 mL of these suspensions was transferred to a 2 mL centrifuge tube and placed inside the induction coil of the AMF source. The samples were heated at a field strength of approximately 55 kA/m and a frequency of 300 kHz for 5 minutes and a Luxtron FOT Lab Kit® (LumaSense Santa Clara, CA) was used to monitor the temperature of the suspension.

The specific absorption rate (SAR) is a calculation that normalizes the heating curve to the concentration of MNPs and indicates the ability of the MNPs to heat. SAR values were calculated for these data using equation 4:

$$\text{SAR} = \frac{\sum c_i m_i}{m} \frac{dT}{dt} \quad (4)$$

where m_i , C_i are the mass and specific heat capacity of component i, respectively, and m is the mass of iron oxide. The ratio dT/dt was determined as the slope of a linear trendline over a 30 second time interval in the initial linear region of the heating curve. The mass of iron oxide, m , for the MF20 and MF5 powders was calculated using results from TGA experiments.

2.12 Cytotoxicity tests

Human A549 alveolar epithelial adenocarcinoma cells were purchased from American Type Culture Collection (Manassas, VA, USA). Cells were cultured in 75 cm² flasks in Dulbecco's modified eagle medium (DMEM) containing 10% FBS, 5% PSTREP, and 1% Fungizone at 37°C in a humidified incubator with 5% CO₂. For viability studies, A549 cells were seeded into 96-well plates at 10,000 cells/cm², placed in an incubator at 37°C and allowed to attach overnight. Cells were subsequently exposed to varying concentrations of MnMs dispersed in DMEM media for 24 hours at 37°C. Following exposure, live cells were stained with calcein AM, which is hydrolyzed to the fluorescent molecule calcein by intracellular esterases, and placed in a Biotek Synergy Mx microplate reader (Biotek, Winooski, VT) to measure the fluorescent intensity. Relative viability was calculated as the ratio of the mean fluorescence intensity to that of the control, which contained media only. For each experiment 10 wells were used for each concentration and experiments were performed in triplicate therefore and the statistical analysis was performed with N=3.

2.13 Statistics

All experiments were performed in at least triplicate. The aerosolization studies were experimentally designed by Design Expert™ 8.0.7.1 software (Stat-Ease Corp., MN, USA). SigmaPlot 12.3 was used for one-way ANOVA tests and when statistically difference was observed a Holm-Sidak test was used to determine any significance in observed data. A p-value of <0.05 was considered statistically significant.

3.0 Results and Discussion

3.1 Physiochemical characterization

Magnetic nanocomposite microparticles (MnMs) were successfully formulated via spray drying and Figure 2 shows representative SEM images of the MnM powders. All particle systems displayed smooth and spherical particle morphologies, which are important for optimal aerodynamic performance. Additionally, these images show a relatively uniform size distribution of the particles, which was confirmed by measuring the geometric diameter of MnM particles with SigmaScan® (Table 2). Representative STEM images of the MnMs show the successful loading of MNPs (Figure 3) via spray drying thereby confirming our hypothesis that composite powders containing novel magnetic nanoparticles can be formulated via spray drying in closed-mode. The presence of MNPs within composite dry powders is evident as areas of higher contrast (brighter regions) and energy dispersive X-ray spectroscopy confirmed these findings (data not shown). The MF20 powder (Figure 3a) shows the highest amount of MNPs and thereby confirms our ability to tailor the amount of MNPs loaded into these novel composites, which would be necessary in the further development of these composites for specific medicinal applications. These images illustrate

the ability to load large quantities of MNPs into spray dried composites and suggest no change in particle shape or morphology. Additionally, these images show that MNPs are dispersed relatively uniformly throughout the MnMs and suggests the spray drying process does not form large MNP aggregates and this was subsequently confirmed with DLS.

DLS, Karl Fisher titration, and TGA were carried out to evaluate the diameters of MNPs released from the composite particles, water content of the powders, and MNP loading in MnMs, respectively. Table 2 summarizes the data from these experiments. DLS was used to measure the hydrodynamic radii of the MNPs before incorporation into MnMs and after release from composites via aqueous dissolution, and Table 2 reports the z-averaged diameter of these systems. The diameter of the iron oxide MNPs before incorporation into MnM composites was 145.7 ± 2.9 nm. After spray drying into MnMs, the diameters of the MNPs in the MF20 and MF5 powders were 159.0 ± 3.8 nm and 144.2 ± 4.5 nm, respectively. These data confirmed minimal differences in the particle diameters of the MNPs released from composites and confirms the absence of agglomerate formation during the formation of MnMs with spray drying. Importantly, these results show the ability to incorporate our MNPs into composite powders for inhalation without losing the advantageous properties associated with their small size. Karl Fisher titration demonstrated that the various MnM powders have low residual water content which is optimal for aerodynamic performance and illustrate that these powders can be stored in such a way to avoid water vapor uptake and the associated complications such as agglomeration due to increased interparticulate interactions. It is interesting to observe that MF20 powders showed a higher mean residual water content than MF5 although this difference was not statistically significantly. Additionally, the variability in the water content was much higher for the MF20 powder and this could be due to residual water present on the MNPs before spray drying. TGA allowed for the quantification of MNP loading in the MnMs and revealed slightly higher loading values in the final spray dried particles than in the initial feed conditions; however, the MNP loading was relatively consistent from batch-to-batch. The increased loading of iron oxide in the final particles is likely due to particles with lower aerodynamic diameters remaining suspended in the airstream longer and therefore continuing to the filter of the spray drying system without impacting and accumulating in the collection vial. The aerodynamic diameter is proportional to the density of the particle [44, 45], and iron oxide has a higher density than D-mannitol. Therefore, particles with less iron oxide will remain suspended in the air longer and be less likely to accumulate in the collection vial. As such, particles containing more MNPs have a higher density and therefore a larger aerodynamic diameter leading to higher probability of collection.

DSC and XRPD were performed to observe thermal phase transitions and crystallinity of the MnM powders, respectively. Figure 4 shows the XRPD diffractograms for the MnMs with important peaks labeled. The representative peaks for iron oxide (Fe_3O_4) are marked with stars and representative peaks for the crystalline polymorphs of d-D-mannitol are marked with a diamond and cross, respectively [46, 47]. The expected peaks for iron oxide are seen in the MF5 and, as expected, become much more intense in the MF20 due to higher MNP loading. The MF0 powder shows peaks from α and β polymorphs of D-mannitol and provides a good reference to see any change in crystallinity due to the incorporation of MNPs. As can be seen, the MF5 and MF20 powders contain the same peaks as MF0 but at

different relative intensities. It is possible that these relative changes could be due to a change in the crystallite size however the width of the peaks remained relatively consistent, and therefore, we suggest that polymorphic changes are primarily responsible for changes in peak intensities. It is observed that there is a change in the ratio of representative peaks for the α and β polymorphs at 2θ degree values of 13.6 and 14.5, respectively. It can be seen that the incorporation of MNPs into the spray dried composites results in a higher β -polymorph character of mannitol in the solid state. This increase in the amount of the β -polymorph demonstrates that the incorporation of iron oxide MNPs results in a polymorphic interconversion between the different crystalline polymorphs of D-mannitol. This also suggests that such phenomena should be closely monitored for future development of powders as conversion between crystalline polymorphs of a given material can lead to changes in its physical properties such as melting point and solubility among others, since polymorphs of the same material have different Gibbs free energy states. DSC thermograms (Figure 5) reveal an endothermic phase transition at approximately 165°C representing the melting point of D-mannitol which is in excellent agreement with its reported melting temperature [47]. These findings indicate that these novel MnMs possess excellent thermal stability and suggest, metaphorically, that these MnMs will “melt in your lungs, not in your hands” (in actuality they will dissolve). These findings are important because they show that the storage of these systems is insensitive to temperature for practical purposes.

3.2 Aerosol performance of MnMs

The MnM powders showed good aerosol performance thereby confirming that they are suitable for dry powder aerosol delivery. The aerosol performance of the MnMs (Figure 6) indicates a large number of the particles being deposited on all stages of the impactor for each powder system thereby illustrating the potential and the versatility of these formulations to reach all regions of the lungs. This would be advantageous for particular lung cancers such as bronchioloalveolar carcinomas, as well as for carcinomas occurring in the upper lung region (e.g. bronchial carcinoma) which can be targeted based on aerodynamic particle size based on the deposition on stage 1. Specifically, particles below 10 microns but greater than 5 microns can effectively target the upper lung region of the bronchi and bronchials [48]. Additionally, these deposition patterns can be altered to shift the deposition pattern to more specific regions by changing processing variables in spray drying as well as by using other excipients [48]. Table 3 summarizes the aerosol performance parameters for each system. The fine particle fraction (FPF) and respirable fraction (RF) values for all of the powders were approximately 30% and 50%, respectively, which are higher than the 10–20% values of currently marketed dry powder inhalation (DPI) pharmaceutical products [48] and comparable to recent reports [49] even in the absence of a lactose carrier. Additionally, these powders all displayed high emitted doses of approximately 90% which demonstrates favorable dispersion efficiency. The mass median aerosol diameter (MMAD) was calculated for the aerosols produced from the MnM powders and all of these values were less than 5 μm suggesting their ability to adequately deposit throughout all regions of the lungs and effectively reach the lower airways. Interestingly, we see similar deposition patterns between MF5 and MF0 while the MF20 powder has slightly higher deposition on some of the earlier stages. This shift in deposition pattern is most likely due to the significant increase in the density of the composite particles as a result of such a

high MNP-loading. Still, all three powders displayed good aerosol performance and these results suggest that these MnMs are suitable for DPI administration and suggest a new administration route for the delivery of iron oxide nanoparticles. Additionally, these findings propose a novel method for incorporating nanomaterials into composites that can subsequently be delivered to the highly permeable tissue of the lungs assuming they can traverse the mucosal lining in regions where it exists.

3.3 AMF heating of MNPs released from MnMs

Representative heating curves for the MnM systems are shown in Figure 7 and these data confirm that the MNPs retained excellent heating capabilities. Experiments were performed at room temperature and therefore illustrate that the temperatures reached for the MF20 powders are well above the hyperthermia range indicating sufficient heat for the intended goals. Additionally, the range of heating between the MF5 and MF20 powders suggests the ability to significantly change heating capability of these powders by altering the MNP loading. The SAR of the iron oxide MNPs before incorporation into MnM composites was 422 ± 18 W/g. After spray drying into MnMs, the SAR values for the MF20 and MF5 powders were 317 ± 51 W/g and 252 ± 53 W/g, respectively. These data illustrate that, although a drop in SAR was seen, the MNPs show excellent heating capability and these SAR values compare favorably with previous reports from our group [50]. High SAR values suggest that relatively low amounts of MNPs would be necessary to achieve elevated temperatures within a biological system.

3.4 In vitro cytotoxicity of MnMs exposed to human lung cell lines

These MnMs deposit effectively *in vitro* on the stages of the NGI which can be modeled for predictive deposition in the alveolar lung region and in the smaller airways (bronchioalveolar region). Hence, it was of interest to test these powders on a human lung cell lines that represent these lung regions for application in lung cancer. Figure 8 shows the results from *in vitro* cytotoxicity studies on the A549 (human lung alveolar epithelial (Type 1) adenocarcinoma) cells. These data reveal a slight decrease in cell viability for higher concentrations of the MnMs indicating moderate cytotoxicity of these materials in this concentration range. Future development of the MNPs incorporated to these MnMs could reduce the cytotoxic effect of these materials by coating them with biocompatible chemical moieties. These preliminary *in vitro* results suggest these materials have the potential to be safe when administered via inhalation aerosol delivery. Additionally, these results indicate that MnMs could provide a novel method of delivering targeted nanotherapy to the lungs in a safe and effective manner.

4.0 Conclusions

Iron oxide MNPs were successfully incorporated into MnMs through spray drying in closed-mode. Physicochemical characterization of these composites revealed the potential to use these particles in the inhalation aerosol treatment of lung cancer via targeted hyperthermia. Aerosol performance studies revealed favorable aerodynamic properties for the MnM powders with FPF and RF values greater than 50% along with ED values around 90%. The MMADs for all aerosols delivered by a currently marketed DPI device were approximately

4.5 μm and the aerosol performance plots suggest the potential for high deposition of these particles throughout the lungs. Heating studies imply that these materials can be remotely heated after formulation and *in vitro* cytotoxicity studies show moderate toxicity on human lung A549 cells. These data provide initial evidence that these materials can potentially be targeted throughout the lung including the upper lung region for application in bronchial lung cancer and also in the mid-to-lower lung regions. Additionally, MnMs possess the ability to release MNPs that remotely heat in the presence of an AMF. These novel composites present an exciting class of materials with many potential applications and pose a promising approach to a novel thermal treatment of the lungs through targeted pulmonary inhalation aerosol delivery of MNPs.

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Figure 1. Schematic representation of the formulation and application of magnetic nanoparticle microcomposites (MnMs). From left to right we see the initial formation of these inhalable dry powders through spray-drying a feed consisting of iron oxide MNPs dispersed in a D-mannitol solution of methanol. Upon collection these materials were loaded into a capsule and placed into a dry powder inhaler for in vitro aerosol dispersion performance studies in order to model their predictive deposition patterns in the lungs. Finally, the heating properties of the MNPs released from these MnMs upon dissolution were verified by exposing them to an alternating magnetic field.

Figure 2.
SEM images of a) MF20, b) MF5, and c) MF0 dry powders. Magnification 5,000 $\times$.

Figure 3.
TEM images of a) MF20, b) MF5, and c) MF0 dry powders.

Figure 4.
XRPD diffractograms for MnM powders.

Figure 5.
DSC thermograms for MnM powders.

Figure 6.
NGL.

Figure 7.
Heating curves for MnMs under AMF exposure

Figure 8.
In vitro cytotoxicity results for human A549 alveolar epithelial adenocarcinoma cells after 24 hours of exposure to MnMs at 37°C.

Table 1

Summary of experimental conditions for inhalable dry powders.

System	Initial MNPs (wt%)	Inlet T (°C)	Outlet T (°C)
MF20	20%	150	67
MF5	5%	150	58
MF0	0%	150	70

Table 2

MnM and MNP diameters, MNP loadings, and water content of MnMs.

System	MNP Diameter (nm)	MnM Diameter (nm)	MNP _{feed} (wt%)	MNP _{actual} (wt%)	Water Content (wt%)
MF20	159.0 ± 3.8	851 ± 505	20	27.8 ± 1.8	4.3 ± 4.0
MF5	144.2 ± 4.5	701 ± 280	5	13.3 ± 1.5	1.2 ± 0.6
MF0	n/a	770 ± 301	0	n/a	1.0 ± 0.6

Table 3

Aerosol performance properties for inhalable MnMs.

	MF20	MF5	MF0
MMAD (μm)	4.5 ± 0.2	4.5 ± 1.0	4.4 ± 1.0
GSD (μm)	2.4 ± 0.1	2.8 ± 0.1	2.7 ± 0.3
FPF (%)	34.3 ± 3.8	31.2 ± 2.0	29.6 ± 6.8
RF (%)	56.5 ± 3.8	50.9 ± 5.6	50.7 ± 6.3
ED (%)	92.0 ± 2.9	92.5 ± 2.9	88.0 ± 6.7